

# Kinetic parameters govern of the uptake of nitrogen forms in 'Paulsen' and 'Magnolia' grapevine rootstocks

Matheus Severo de Souza Kulmann<sup>a</sup>, Paula Beatriz Sete<sup>b</sup>, Betania Vahl de Paula<sup>a</sup>,  
Lincon Oliveira Stefanello<sup>a</sup>, Raíssa Schwalbert<sup>c</sup>, Raí Augusto Schwalbert<sup>d</sup>,  
Wagner Squizani Arruda<sup>a</sup>, Gabriel Alberto Sans<sup>a</sup>, Carolina Flogiarini Parciannelo<sup>a</sup>,  
Fernando Teixeira Nicoloso<sup>c</sup>, George Wellington Bastos de Melo<sup>e</sup>, Henrique Pessoa dos Santos<sup>e</sup>,  
Gustavo Brunetto<sup>a,\*</sup>

<sup>a</sup> Soil Science Department of Federal, University of Santa Maria (UFSM), 97105-900, Santa Maria, RS, Brazil

<sup>b</sup> Federal University of Santa Catarina, Rodovia Admar Gonzaga, 1346, CEP 88034-001, Florianópolis, SC, Brazil

<sup>c</sup> Agronomy Department of Federal, University of Santa Maria (UFSM), 97105-900, Santa Maria, RS, Brazil

<sup>d</sup> Agricultural Engineering Department of Federal, University of Santa Maria (UFSM), 97105-900, Santa Maria, RS, Brazil

<sup>e</sup> National Research Center of Grape and Wine, Brazilian Agricultural Research Corporation (Embrapa), 95700-000, Bento Gonçalves, RS, Brazil

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## ABSTRACT

Grapevine rootstocks are selected according to graft compatibility, pathogen tolerance and water stress. However, morphological and kinetic parameters of nutrient absorption, such as nitrate ( $NO_3^-$ ) and ammonium ( $NH_4^+$ ), usually are not considered. The study aimed to select, based on morphological and kinetic absorption parameters, grapevine rootstocks with greater efficiency of N-forms absorption. The study was performed with two grapevine rootstocks ('Paulsen 1103' and 'Magnolia'), grown in Hoagland nutrient solution in a greenhouse. After 21 d, they were placed in 0.05 mol L<sup>-1</sup> CaSO<sub>4</sub> solution for 20 d to exhaust nutrient internal reserves. The rootstock with highest  $NO_3^-$  and  $NH_4^+$  uptake efficiency was 'Paulsen 1103', since it presented lower  $C_{min}$  and  $K_m$  values in the two N forms, allowing N extraction by roots, even in low concentrations in solution, due to the higher transporter affinity for  $NO_3^-$  and  $NH_4^+$  in roots from rootstock. 'Paulsen 1103' rootstock also presented better photosynthetic performance and root development, collaborating for greater growth and mass production than 'Magnolia' rootstock. On the other hand, 'Magnolia' rootstock showed higher  $NO_3^-$  and  $NH_4^+$   $C_{min}$  and  $K_m$  values than 'Paulsen' rootstock, which shows lower transporters affinity for these ions. Results demonstrate the importance of the use of  $NO_3^-$  and  $NH_4^+$  uptake kinetic parameters in grapevine rootstock selection programs, since these may serve as indicator of plant's ability to absorb  $NO_3^-$  and  $NH_4^+$  in low concentrations.

## 1. Introduction

Grapevine rootstocks determine strength of the crown, productivity and grape composition, mainly due to rootstock ability to regulate nutrients absorption and transport (Lecourt et al., 2015; Zamboni et al., 2016). Some rootstocks, such as 'Paulsen 1103', are widely used due to its efficient use of soil water. Because of it, they may be more adaptable to areas with water limitation. It is also reported their medium tolerance to acidic soils and moderate to saline soils, and that their cultivation in these environments does not compromise grape development and quality (Tsegay et al., 2014). Other important factor of its cultivation is its high resistance to drought, nematodes and Phylloxera

(*Daktulosphaira vitifoliae*) (Serra et al., 2014; Contreras et al., 2019). The 'Magnolia' rootstock is known for its great potential to be used in areas with frequent rainfall and high temperatures, due to its resistance to shoot fungal diseases, such as mealybug (Hemiptera: Pseudococcidae), root fungal diseases, such as *Eurhizococcus brasiliensis* (Hemiptera: Margarodidae) (Botton and Colleta, 2010). However, in the grapevine rootstocks selection, nutrient uptake efficiency, such as nitrogen (N), which is the element that most impacts productivity, grape composition and its sub-products, such as wines and juices, is not considered (Spyay et al., 1994; Brunetto et al., 2016; Zamboni et al., 2016). Based on this, the selection of grapevine rootstocks with higher N uptake efficiency, may contribute to future breeding programs and this might impact on

\* Corresponding author.

E-mail address: [brunetto.gustavo@gmail.com](mailto:brunetto.gustavo@gmail.com) (G. Brunetto).

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higher grape yields and quality in the future.

The N is a nutrient required by plants in greater quantity, being constituent of amino acids, protein, nucleic acids, chlorophyll, phytohormones, among others (Tomasi et al., 2015; Marschner, 2012). The N uptake by plants is preferably in nitrate ( $\text{NO}_3^-$ ) or ammonium ( $\text{NH}_4^+$ ) mineral forms, and is mediated by carrier proteins present in plasma membrane of epidermal cells and root cortex (Marschner, 2012). These transporters may present different affinities for N ( $\text{NO}_3^-$  and  $\text{NH}_4^+$ ) forms, being classified as high or low affinity transporters, HATS and LATS respectively, or they may present duality. In general, HATS operate at lower concentrations (less than  $0.5 \text{ mmol L}^{-1}$ ), whereas LATS act at higher ions concentrations in solution (greater than  $0.5 \text{ mmol L}^{-1}$ ). Thus, it is expected that plants adapted to a low nutrients availability scenarios trigger high affinity systems, especially in the case of N (Castro-Rodríguez et al., 2017; Xuan et al., 2017).

Light signals reception by shoot and transmission to other organs regulate root system development, which contributes to water and nutrients absorption, as N, by plants (Lee et al., 2016). This occurs through of the signaling molecules transport from the shoot to roots, as well as important photoreceptors, such as phytochrome A (phyA) activation, which transfers auxin from the shoot to root system, stimulating root production, positively influences  $\text{NO}_3^-$  and  $\text{NH}_4^+$  uptake by the plant (Lee et al., 2016; Xuan et al., 2017). The N assimilated by the plant contributes to important physiological mechanisms, especially in proteins and enzymes production, which can trigger an increase in PSII maximum quantum yield (Fv/Fm), and in electron transport (ETRm), resulting in a higher  $\text{CO}_2$  liquid assimilation rate. Under high luminosity conditions, the enzyme responsible for  $\text{NO}_3^-$  reduction in the assimilation process is activated, allowing process continuity and stimulating  $\text{NO}_3^-$  absorption (Marschner, 2012).

The  $\text{NO}_3^-$  and  $\text{NH}_4^+$  absorption is also determined by kinetic parameters. These include the maximum absorption rate ( $V_{\max}$ ), Michaelis-Menten constant ( $K_m$ ), minimum concentration ( $C_{\min}$ ) and influx (I) (Martinez et al., 2015). The  $V_{\max}$  corresponds to saturation of transport sites of root cells membrane by absorbed ions occurs.  $C_{\min}$  refers to the minimum nutrients concentration in solution so that roots activate the onset of absorption. The  $K_m$  describes the ion transporter affinity by its ion to be transported. The lower the  $K_m$  value, the higher the ion affinity by its transport sites. The I represent the liquid absorption rate of the ion in a concentration solution (Martinez et al., 2015). Kinetic parameters are used to elucidate which plants are most efficient in nutrient uptake and which are best suited to soils with low natural fertility, such as soils with low organic matter content and, consequently, low native N availability. On the other hand, it also allows to identify more demanding plants in N, which may require higher N amounts during its development, which can be made available through N fertilizer applications, in order to supply plant N demand.

Kinetic parameters related to absorption of N forms have already been reported for annual species such as rice (Araújo et al., 2015), corn (Horn et al., 2006), barley (Glass et al., 1990) and Chinese kale (Song et al., 2016). However,  $\text{NO}_3^-$  and  $\text{NH}_4^+$  absorption rates by grapevine rootstocks roots, such as 'Paulsen 1103' and "Magnolia", are not sufficiently known. Obtained information may contribute to grapevine rootstocks cultivars selection with greater nutrient absorption capacity and in the zoning of cultivars more adapted to each region soil conditions, contributing to increase grape productivity and quality. This study aimed to select grapevine rootstocks with greater efficiency in N absorption, according to the kinetic parameters related to the absorption of N forms.

## 2. Material and methods

### 2.1. Vegetal material and treatments

The experiment was conducted from November to December 2017, in the greenhouse of Soils Department in the Federal University of Santa

Maria (UFSM), Santa Maria, Rio Grande do Sul, Brazil. During the experiment, the greenhouse was maintained with a  $25^\circ\text{C}$  average temperature and 60 % relative humidity. Two grapevine rootstocks (hy-pobiote), produced from herbaceous branches from 'Paulsen 1103' and 'Magnolia' cultivars, were used. Branches with 10 cm length containing three upper buds and with complete leaves were collected from adult matrices for the preparation of cuttings. The lower part of branches was immersed in hydroalcoholic solution containing indol-butyric acid  $3.0 \text{ mg L}^{-1}$  for five min. Subsequently, branches were packed in culture containers using vermiculite as substrate. Branches remained 60 d in an intermittent nebulization chamber with  $25^\circ\text{C}$  average temperature and 70 % average humidity. Branches were previously selected according to mean roots number and were transplanted into 500 mL disposable plastic cups containing commercial substrate based on charcoal rice husk and vermiculite (30:70). After that, five replicates of each grapevine cultivar containing 10–15 leaves, homogenous height and vigor were transferred to pots with 8 L capacity, containing 5 L Hoagland nutrient solution at 50 % total strength (Jones, 1983), with the following composition: ( $\text{mg L}^{-1}$ )  $\text{N-NO}_3^- = 98$ ,  $\text{N-NH}_4^+ = 7$ ,  $\text{P} = 15.5$ ,  $\text{K} = 117$ ,  $\text{Ca} = 80$ ,  $\text{Mg} = 24.3$ ,  $\text{S} = 35$ ,  $\text{Fe-EDTA} = 2.5$ ,  $\text{Cu} = 0.01$ ,  $\text{Zn} = 0.075$ ,  $\text{Mn} = 0.25$ ,  $\text{B} = 0.25$  and  $\text{Mo} = 0.005$ . In a hydroponic system with continuous aeration, the plants were fixed in the vessels through styrofoam blades with a central hole, which prevented solar radiation entry and decreased solution evaporation. Plants were subjected to an acclimation period in nutrient solution for 21 d, solution was changed every 5 d and pH adjusted to  $6.0 \pm 0.2$  with  $1 \text{ mol L}^{-1}$  HCl or  $1 \text{ mol L}^{-1}$  NaOH. Experimental design was completely randomized, with five replicates per treatment, each plant being considered an experimental unit.

### 2.2. Liquid absorption kinetics of $\text{NO}_3^-$ and $\text{NH}_4^+$

After the 21 d acclimation period, the kinetic parameters ( $V_{\max}$ ,  $K_m$  and  $C_{\min}$ ), and  $\text{NO}_3^-$  and  $\text{NH}_4^+$  absorption rates of grapevine rootstocks were determined by the methodology proposed by Claassen and Barber (1974) with adaptations for completely deplete N internal reserves in plants. Thus, grapevine plants were transferred to vessels with 8 L capacity, containing 5 L of  $0.05 \text{ mol L}^{-1}$   $\text{CaSO}_4$  solution for 20 d. Subsequently, the  $\text{CaSO}_4$  solution from each vessel was replaced with Hoagland's nutrient solution at 50 % total strength, and waited 1 h for the reestablishment of steady-state absorption conditions required for kinetic model application (Paula et al., 2018). After 1 h, Hoagland's nutrient solution at 50 % total strength was again replaced for a new solution containing the same concentration. Immediately, 10 mL solution was collected (time 0). Then 10 mL of solution were collected every 6 h until the 30 h period was reached. After 30 h, 10 mL of solution was collected every 3 h, up to 54 h. And, in 54–65 h the period, 10 mL of solution was collected every 1 h. Thus, the solution was collected for 65 h. Collected solutions were stored and frozen at  $-10^\circ\text{C}$  for further analysis.

### 2.3. Photosynthetic parameters

Photosynthetic parameters were measured in the third fully expanded leaf of the stem, after the  $\text{CaSO}_4$  exposure period of plants, using the portable Infra Red Gas Analyzer (IRGA) (LI-6400 XT, LI-COR, Inc., Lincoln, NE, USA). The photosynthetic radiation used was  $1500 \mu\text{mol m}^{-2} \text{ s}^{-1}$  and  $\text{CO}_2$  concentration of  $400 \mu\text{mol mol}^{-1}$ . Measurements were performed in the morning period, between 8:00 and 10:00. Were evaluated:  $\text{CO}_2$  liquid assimilation rate ( $A - \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ); water vapors stomatal conductance ( $G_s - \text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ); intercellular  $\text{CO}_2$  concentration ( $E - \text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ); water use efficiency ( $\text{WUE} - \text{mol CO}_2 \text{ mol H}_2\text{O}^{-1}$ ), obtained by the relation between amount of  $\text{CO}_2$  fixed in photosynthesis and amount of transpired water; and rubisco carboxylation efficiency ( $A/\text{Ci}$ ), obtained by the relation between amount of  $\text{CO}_2$  fixed in photosynthesis and internal

CO<sub>2</sub> concentration.

#### 2.4. Chlorophyll *a* fluorescence evaluation

Chlorophyll *a* fluorescence emission was analyzed after exposure period of plants in CaSO<sub>4</sub> solution, performed in the first fully expanded leaf in three plants per treatment, on a sunny day, during the morning period, between 8:00 and 9:30 (Souza et al., 2013), using portable light-modulated fluorometer (Junior-Pam Chlorophyll Fluorometer Walz Mess-und-Regeltechnik, Heinz Walz GmbH, Effeltrich, Germany). Prior to measurements, leaves were preadapted to dark by wrapping in foil for 30 min in order to measure the initial fluorescence (Fo). Subsequently, samples were subjected to a pulse of saturating light (10.000 μmol m<sup>-2</sup> s<sup>-1</sup>) for 0.6 s, thus determining the maximum fluorescence (Fm). The maximum PSII quantum yield (Fv/Fm) was obtained by the ratio between variable fluorescence (Fv = Fm-Fo) and maximum fluorescence. The electron transport rate (ETRm) was evaluated using the induction curve fluorescence.

#### 2.5. Plant collection and analysis of N concentration in tissue and solution

After 65 h of evaluation, plants were removed from container containing nutrient solution and were fractionated in leaves, stems and roots. Nutrient solution remaining volume in each vial was measured using graduated cylinder. Roots and shoot fresh matter was weighed using a digital scale (Bel Engineering, Precision balance L, Monza, Milan, Italy). Organs were oven dried with forced air at 65 °C until constant mass. They were then weighed for dry matter determination. Immediately after weighing, they were milled in Willey mill with 2 mm sieve. Subsequently, to determine N concentration in the organs, finely ground subsamples were dried at 65 °C using an elemental analyzer (FlashEA 1112, Thermo Electron Corporation, Milan, Italy).

After 65 h of nutrient solution sampling, NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> were determined by colorimetric methods similar to those described by Künsch et al. (1977). It was performed using a Segmented Flow Analyzer System (SAN<sup>++</sup> System, Skalar, Netherlands).

#### 2.6. Root system morphology

Roots morphological characterization was obtained from digitalized images using an EPSON Expression 11000 scanner equipped with additional light (TPU) with a 600 DPI resolution. The scanned images were used to determine root morphological traits using WinRHIZO Pro software (Regent Instrument Inc., Quebec City, Quebec, Canada). Total root length (cm plant<sup>-1</sup>), root surface area (cm<sup>2</sup> plant<sup>-1</sup>), root volume (cm<sup>3</sup> plant<sup>-1</sup>), root mean diameter (mm), and relative distribution of root length in diameter classes (%) of 0 < L ≤ 0.2; 0.2 < L ≤ 0.45; 0.45 < L ≤ 0.75; 0.75 < L ≤ 1.5; L > 1.5, were measured.

#### 2.7. Calculations and statistical analysis

Data on kinetic parameters (V<sub>max</sub> and K<sub>m</sub>) were calculated according to NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> concentrations in Hoagland nutrient solution, initial and final solution volumes in the vessels and roots fresh matter values using the software Influx. To obtain C<sub>min</sub> value, NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> concentrations present in nutrient solution, corresponding to the time of 65 h of evaluation, were used. To obtain the liquid influx (IL) value, we used Eq. (1) proposed by Michaelis-Menten and modified by Nielsen and Barber (1978).

$$IL = \left[ \frac{V_{max} \times (C - C_{min})}{K_m + (C - C_{min})} \right] \quad (1)$$

where: V<sub>max</sub> is membrane transporters maximum absorption rate; C is concentration present in solution at collection time; C<sub>min</sub> is the minimum concentration at 65 h period and K<sub>m</sub> refers to transporter

affinity coefficient by solute.

Data were processed and statistically analyzed using R statistical environment (R Development, Core Team, 2019). Obtained results were submitted to analysis of variance and, when significant, were compared by Student's *t*-test at 5 % probability of error (*P* < 0.05).

Additionally, data were also submitted to principal component analysis (PCA), using Canoco software version 4.5 (Ter Braak and Smilauer, 2002). PCA is generally used to find the weights of each variable in order to maximize the variance among sampling points (Ortega et al., 1999). The PCA is performed according to a set of principal components (PC1 and PC2 in this case), which are composed of a set of standardized orthogonal linear combinations that together explain the variance of original data. This analysis allows us to identify more complex interactions among the evaluated variables and to identify those with greater contribution to the differences among our treatments.

### 3. Results and discussion

#### 3.1. Plant growth and root morphology

'Paulsen 1103' rootstock had the highest values of height increase and diameter, leaves dry matter production, stems and total dry matter; N concentration in roots and greater N accumulation in leaves, stems and roots (Table 1). The highest N accumulation in organs as leaves, stem and roots possibly occurred due to the intense cellular division and elongation in these organs, which was reflected in higher growth and total dry matter production, increasing nutrients demand, among them N (Lee et al., 2016; Zufferey et al., 2015; Paula et al., 2018). On the other hand, 'Magnolia' rootstock showed the highest root/shoot ratio (R/S) value, since it presented lower shoot growth over time, and this can be observed by the lower values of increase in height, stem diameter and shoot dry matter production (Table 1). This may be a result of 'Magnolia' rootstock have invested many photoassimilates for root growth in relation to shoot, for example, in order to absorb more N from solution (Lee et al., 2016; Xuan et al., 2017). In addition, the root/shoot ratio (R/S) value determined by the smaller shoot amount, implies in a smaller light interception capacity, which can cause less C fixation (Canarini et al., 2019; Greer, 2018). Therefore, the rootstock tends to have a lower photoassimilates concentration required in NO<sub>3</sub><sup>-</sup> reduction process in roots, which may cause NO<sub>3</sub><sup>-</sup> absorption inhibition (Marschner, 2012).

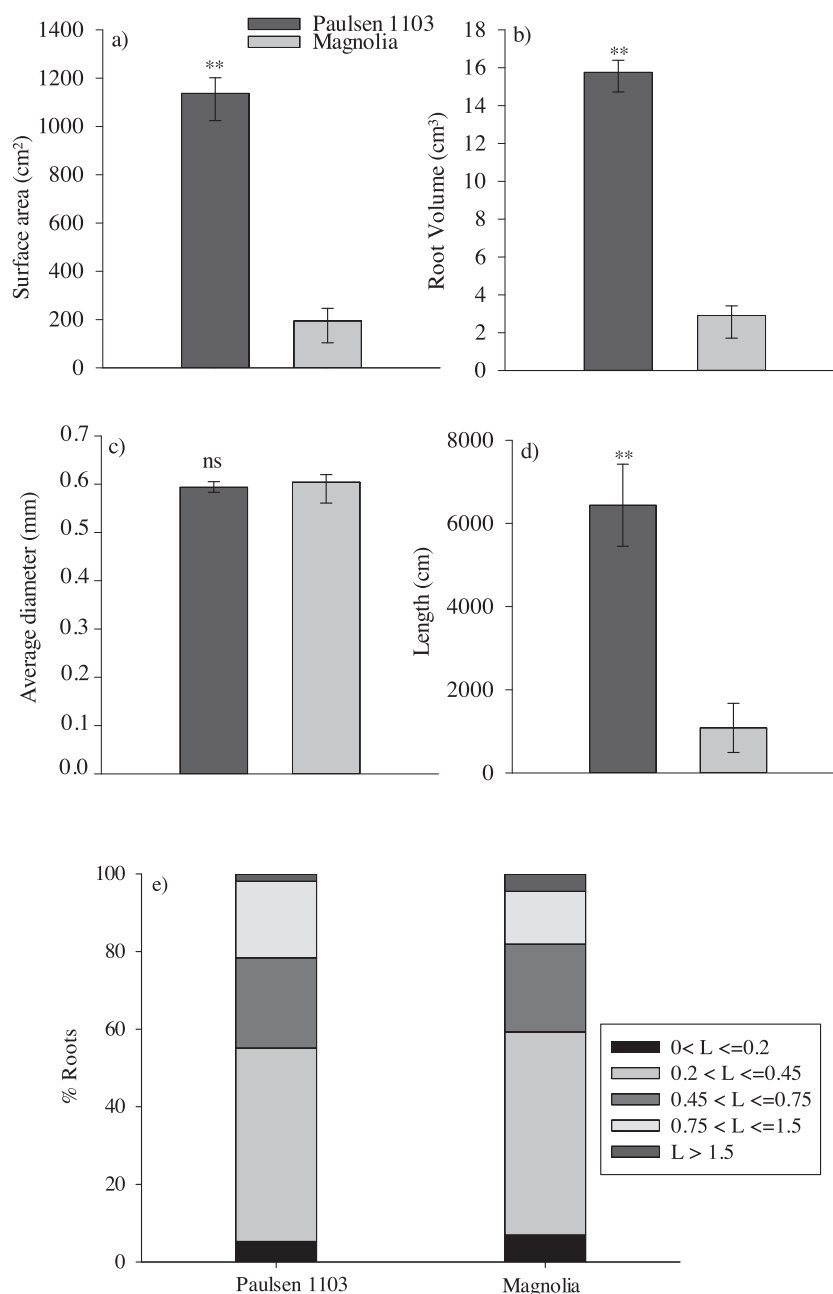
'Paulsen 1103' rootstock had the highest length, surface area and root volume values (Fig. 1a, b, d). From this, roots are expected to

**Table 1**

Morphological parameters, accumulation and total N content in organs of 'Paulsen 1103' and 'Magnolia' rootstocks, grown in Hoagland nutrient solution after 20 d of reduced internal nutrient reserves.

Parameters	'Paulsen 1103'	'Magnolia'
Increase in height (cm)	107.56 ± 9.88 **	80.12 ± 2.24
Increase in stem diameter (cm)	0.73 ± 0.06 **	0.55 ± 0.06
Leaf dry matter (g)	7.60 ± 0.46 **	5.67 ± 0.45
Stem dry matter (g)	9.11 ± 0.49 **	6.43 ± 0.68
Root dry matter (g)	2.34 ± 0.11 <sup>ns</sup>	2.29 ± 0.50
Total dry matter (g)	19.05 ± 0.71 **	14.39 ± 0.16
Root/shoot ratio	0.58 ± 0.02 **	0.82 ± 0.04
Total N in leaf (%)	1.49 ± 0.21 <sup>ns</sup>	1.57 ± 0.30
Total N in stems (%)	0.46 ± 0.05 <sup>ns</sup>	0.46 ± 0.09
Total N in roots (%)	1.00 ± 0.02 *	0.84 ± 0.05
N accumulated in leaf (g organ <sup>-1</sup> )	10.51 ± 0.77 *	8.19 ± 1.04
N accumulated in stems (g organ <sup>-1</sup> )	4.11 ± 0.44 **	3.30 ± 0.35
N accumulated in roots (g organ <sup>-1</sup> )	2.34 ± 0.16 *	1.70 ± 0.29

Means ± SE followed by statistical significance (\* = Significant by Student's *t*-test (*p* < 0.05); \*\* = Significant by Student's *t*-test (*p* < 0.01); <sup>ns</sup> = not significant).



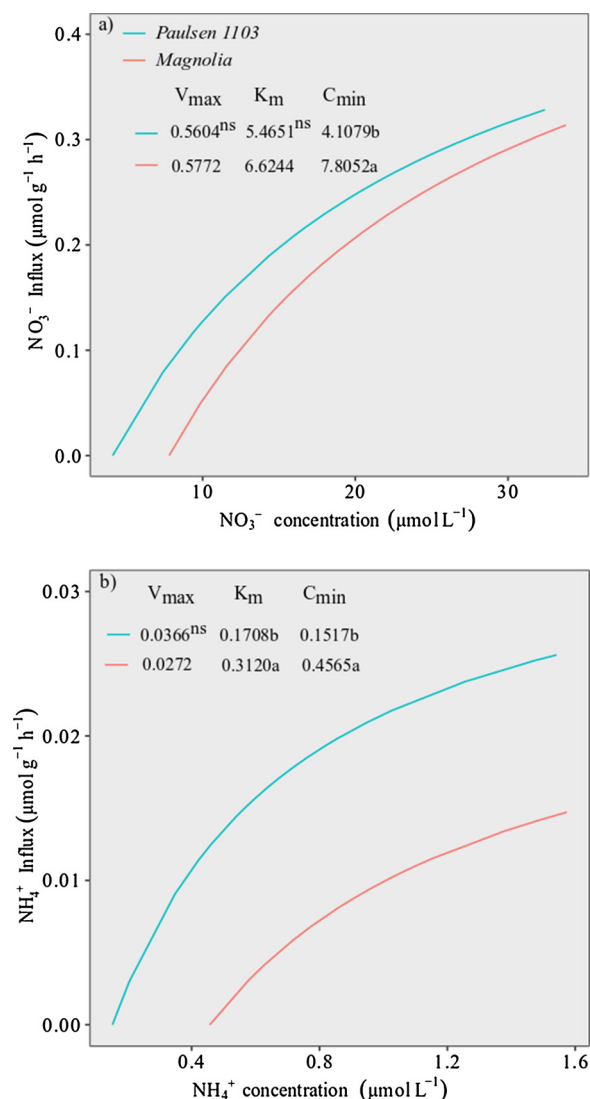
**Fig. 1.** Surface area (a), root volume (b), average diameter (c), length (d) and percentage distribution of roots for each diameter range (e) of 'Paulsen 1103' and 'Magnolia' rootstocks, grown in Hoagland nutrient solution after 20 d of reduced internal nutrient reserves. Vertical bars indicate mean and relevant asterisk at the top indicate statistical significant by Student's *t*-test. (\* = Significant by Student's *t*-test ( $p < 0.05$ ); \*\* = Significant by Student's *t*-test ( $p < 0.01$ ); ns = not significant).

exploit a larger environmental volume (eg. soil solution), potentiating the higher water and nutrients absorption rate, as N, especially by fine roots ( $< 1.5$  mm in diameter), which were predominant in 'Paulsen 1103' rootstock root system (Fig. 1e) (Brunner and Godbold, 2007; Xia et al., 2010; Yan et al., 2017). The highest fine roots abundance in the root system may have occurred due to the higher solar radiation uptake made possible by higher shoot dry matter production (Table 1). Thus, light signals are emitted from shoot towards the roots, activating the emission of new roots, predominantly fine ones, of diameter smaller than 2.0 mm and/or root hairs (Lee et al., 2016). Increases in this root class contributes positively to N uptake, since, by modifying root system architecture, it facilitates access to water (Skaggs and Shouse, 2008) and nutrients (Lambers et al., 2006) reducing the effects of abiotic stress (George et al., 2014; Topp et al., 2016; White et al., 2013).

### 3.2. Kinetic parameters of $\text{NO}_3^-$ and $\text{NH}_4^+$ uptake

The 'Paulsen 1103' rootstock showed the lowest  $C_{\min}$  value for  $\text{NO}_3^-$  when compared to 'Magnolia' rootstock (Fig. 2a). This may be a result from the highest values of length, surface area and root volume (Fig. 1a, b, d) presented by 'Paulsen 1103' rootstock, consequently contributing to water and nutrients absorption (Lambers et al., 2006; Raven et al., 2018; Canarini et al., 2019). The  $C_{\min}$  results suggest that 'Paulsen 1103' rootstock has a higher ability to absorb  $\text{NO}_3^-$ , even at low concentrations in the environment. In this way, 'Paulsen 1103' rootstock has the ability to access  $\text{NO}_3^-$  in a greater number of absorption sites per root unit compared to 'Magnolia' rootstock (Tomasi et al., 2015; Batista et al., 2016). This indicates that 'Paulsen 1103' rootstock can be cultivated in solution or soil with lower  $\text{NO}_3^-$





**Fig. 2.** Influx rates and kinetic parameters of  $\text{NO}_3^-$  (a) and  $\text{NH}_4^+$  (b) uptake of 'Paulsen 1103' and 'Magnolia' rootstocks grown in Hoagland nutrient solution after 20 d of reduced internal nutrient reserves. Means with different letters indicate significant differences by Student's *t*-test ( $P < 0.05$ ).

availability, which is desired, since the lower the N fertilizers doses to be applied, the less the fertilizer acquisition expense and contamination potential of surface and subsurface waters adjacent to areas under grapevines (Bindraban et al., 2015; Bednorz et al., 2016). However,  $\text{NO}_3^-$   $V_{\max}$  values obtained for both 'Paulsen 1103' and 'Magnolia' rootstocks, did not differ statistically (Fig. 2a). This suggests that both rootstocks have the same nutrient uptake condition in solution when all transporters sites present in root cells membranes are saturated (Yang et al., 2007; Martinez et al., 2015).

Possibly, a high  $\text{NO}_3^-$  affinity transport system (HATS) is activated in the roots membranes of 'Paulsen 1103' rootstock, whereas for 'Magnolia' rootstock, the active system must be of low affinity (LATS), being each mediated by more than one type of protein. The molecular basis of these absorption systems is documented for *Arabidopsis* (Doddema and Telkamp, 1979; Dechorgnat et al., 2010), which shows the predominance of NRT1 and NRT2 transporters families, respectively, to LATS and HATS, with the exception of NRT1.1, a double affinity transporter. In addition, there is also the action of the protein with different enzymatic kinetics and efflux controlled by NAXT1 transporters, belonging to the large NRT1/Peptide Transporter (NRT1/PTR) family, being characterized as a key protein in the passive  $\text{NO}_3^-$

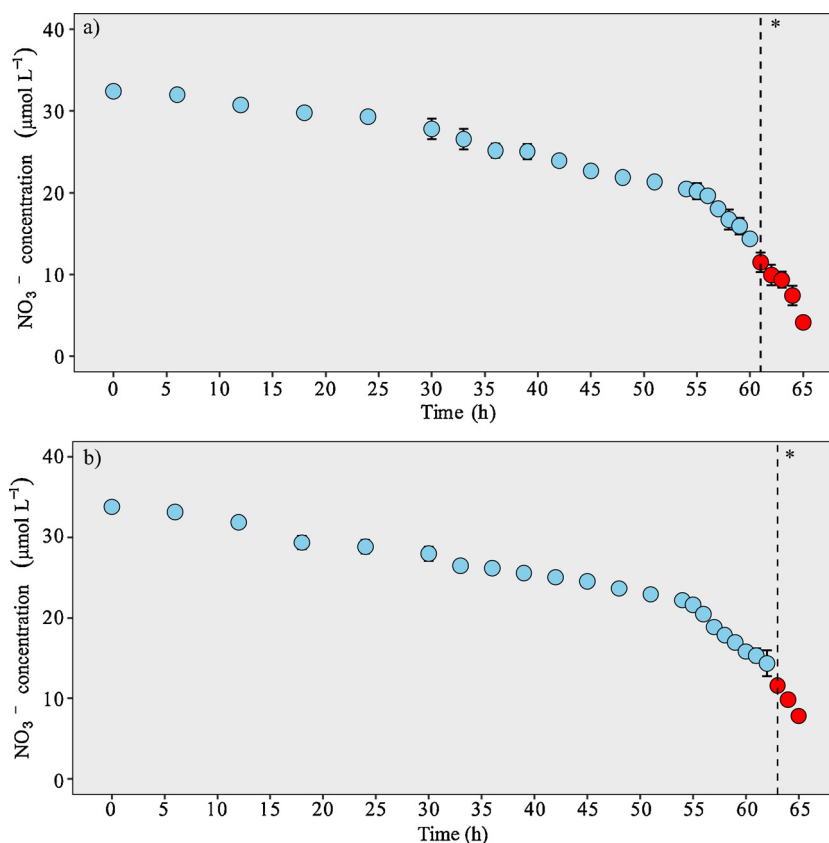
efflux to the cell exterior (Glass, 2003; Segonzac et al., 2007; Tomasi et al., 2015). It is believed that,  $\text{NO}_3^-$  absorption, when carried out by HATS, occurs in a 0–0.5 mmol L<sup>-1</sup> range, allowing two high affinity transport systems to be activated: (i) a constitutive one (cHATS), where  $K_m$  occurs between 6–20  $\mu\text{mol L}^{-1}$ , as possibly verified in 'Magnolia' rootstock, with  $K_m$  ( $> 6 \mu\text{mol L}^{-1}$ ); (ii) and another one  $\text{NO}_3^-$  induced system (iHATS), with  $K_m$  in 20–100  $\mu\text{mol L}^{-1}$  range (Tomasi et al., 2015). On the other hand, 'Paulsen 1103' rootstock operates in a transport system (cHATS), but with a higher  $\text{NO}_3^-$  affinity than 'Magnolia' rootstock, reaching  $K_m$  ( $< 6 \mu\text{mol L}^{-1}$ ).

'Paulsen 1103' rootstock also showed the lowest  $K_m$  and  $C_{\min}$  values for  $\text{NH}_4^+$  uptake compared to 'Magnolia' rootstock (Fig. 2b). Thus, as for  $\text{NO}_3^-$  uptake,  $\text{NH}_4^+$  uptake was directly influenced by root morphological parameters, which were superior in 'Paulsen 1103' rootstock compared to 'Magnolia' rootstock (Fig. 1). In addition to morphological parameters,  $\text{NH}_4^+$  uptake kinetic parameters presented by 'Paulsen 1103' rootstock possibly provided higher  $\text{NH}_4^+$  uptake due to lower  $K_m$  values, reflecting the higher affinity of  $\text{NH}_4^+$  transporters when compared to 'Magnolia' rootstock. Possibly, plants from 'Paulsen 1103' rootstock provided the high affinity  $\text{NH}_4^+$  transporters activation (lower  $K_m$ ). 'Paulsen 1103' rootstock possibly operates in a high affinity transport system, allowing  $\text{NH}_4^+$  absorption even when the cation occurs at very low concentrations (Couturier et al., 2007; Li et al., 2012). The Ammonium transporters (AMTs) are proteins responsible for  $\text{NH}_4^+$  transport on plants plasma membrane, providing the main route for  $\text{NH}_4^+$  influx in roots (Xuan et al., 2017; Castro-Rodríguez et al., 2017). The AMT1  $\text{NH}_4^+$  transporters subfamily is responsible for the high affinity transport of  $\text{NH}_4^+$  while AMT2 subfamily, by the low affinity transport (Castro-Rodríguez et al., 2017). The highest I of  $\text{NH}_4^+$  values were observed in 'Paulsen 1103' rootstock (Fig. 2b), explaining the higher N accumulation in leaves and stem and, consequently, higher dry matter production of these organs, besides the greater height and diameter increase than observed in 'Magnolia' rootstock (Table 1). The higher  $\text{NH}_4^+$  uptake may have generated a lower energy expenditure on the rootstock during assimilation process, since it is more advantageous for the plant to absorb  $\text{NH}_4^+$  in relation to  $\text{NO}_3^-$  since reduction is unnecessary (Castro-Rodríguez et al., 2017; Lang et al., 2018; Zhou et al., 2015).

### 3.3. Uptake evaluation of $\text{NO}_3^-$ and $\text{NH}_4^+$ along kinetic gait time

The  $\text{NO}_3^-$  uptake kinetic gait showed that 'Paulsen 1103' and 'Magnolia' rootstocks behave similarly until close to 50 h of evaluation (Fig. 3). This decay behavior of  $\text{NO}_3^-$  in solution occurs tenuously and then, after that,  $\text{NO}_3^-$  decay in solution occurs in a sinuous way. Responses with this decay format have already been reported in other N uptake studies (Yang et al., 2007; Pii et al., 2019). However, the marked  $\text{NO}_3^-$  decays in solution for 'Paulsen 1103' rootstock occurred within 50 h of evaluation, whereas in 'Magnolia' rootstock it occurred only in 54 h of evaluation. This shows that rootstocks have distinct root morphological characteristics, resulting in differences in  $\text{NO}_3^-$  uptake mechanisms. Possibly, roots cells plasma membranes of 'Paulsen 1103' and 'Magnolia' rootstocks act through LATS, up to 50 and 54 h of evaluation, respectively. After this,  $\text{NO}_3^-$  concentration in solution decreases and uptake is initiated through another system (HATS) until it reaches  $C_{\min}$ . In this way, 'Magnolia' rootstock absorbed  $\text{NO}_3^-$  in a more subtle way compared to 'Paulsen 1103', since they reached  $C_{\min}$  at 63 and 61 h from the beginning of evaluation, respectively. This result proves that grapevine rootstocks vary in  $\text{NO}_3^-$  absorption intensity, and this is probably related to each rootstock genetic characteristics (Tomasi et al., 2015; Kiba and Krapp, 2016).

Initially,  $\text{NH}_4^+$  uptake gait results demonstrated that roots of both rootstocks absorbed  $\text{NH}_4^+$  intensely up to 24 h of evaluation (Fig. 4). Shortly thereafter, there was a decrease in  $\text{NH}_4^+$  uptake up to 50 h of evaluation for both rootstocks. This could possibly be due to the low induction of proteins that act in  $\text{NH}_4^+$  transport to the interior of roots



**Fig. 3.** Concentration of  $\text{NO}_3^-$  in nutrient solution, cultivated with 'Paulsen 1103' (a) and 'Magnolia' (b) rootstocks grown in Hoagland nutrient solution after 20 d of reduced internal nutrient reserves. \* Time reaching lowest concentration  $p < 0.05$ .

plasma membrane of rootstocks, being the main  $\text{NH}_4^+$  influx route. This occurs mainly when there is saturation of  $\text{NH}_4^+$  uptake sites, elucidating an initial stage of  $\text{NH}_4^+$  uptake by rootstocks, performed by two  $\text{NH}_4^+$  transport mechanisms. Probably, an  $\text{NH}_4^+$  absorption mechanism of low affinity occurs, up to 24 h, which is saturated until near to 50 h. After this, another  $\text{NH}_4^+$  absorption mechanism is activated, this one with high affinity for the ion. In this way,  $\text{NH}_4^+$  uptake by transporters decreases until reaching  $C_{\min}$ . 'Paulsen 1103' and 'Magnolia' rootstocks reached  $C_{\min}$  at 64 and 65 h of evaluation, respectively (Fig. 4).

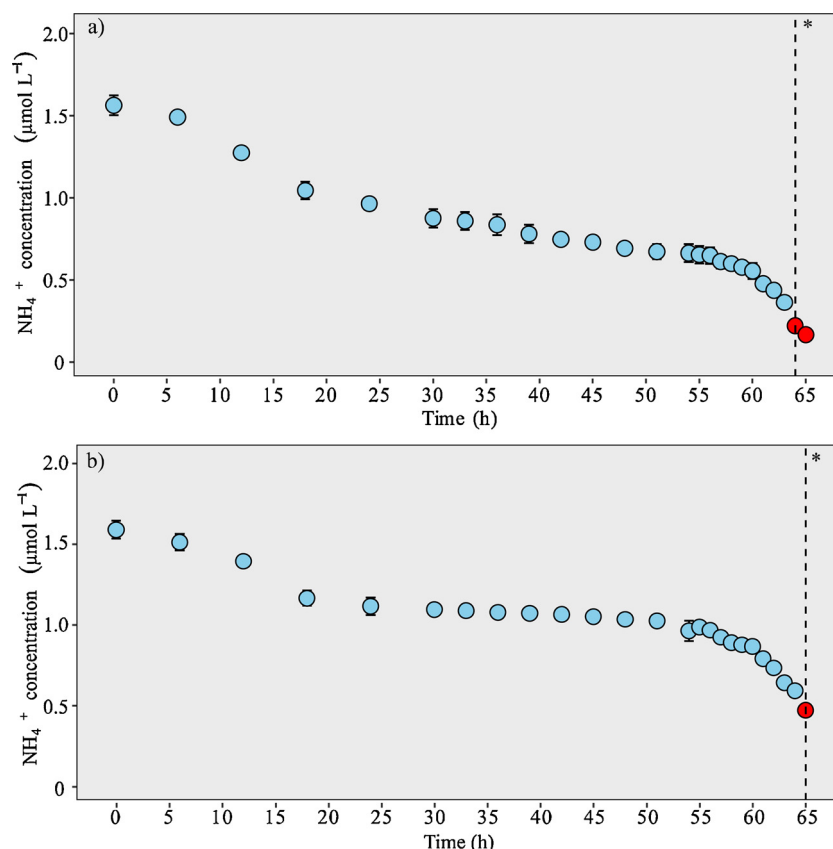
It should be noted that both 'Paulsen 1103' and 'Magnolia' rootstocks absorb  $\text{NO}_3^-$  and  $\text{NH}_4^+$  continuously over the 65 h evaluated and, only in the last evaluation h, did they reach  $\text{NO}_3^-$  and  $\text{NH}_4^+$   $C_{\min}$ . This demonstrates the relevance of more spaced solution collections in initial absorption hours of these plants, as well as an evaluation time greater than 5 h, as used for Chinese kale (Song et al., 2016), 8 h for rice (Araújo et al., 2015) and 24 h for corn (Horn et al., 2006). In addition, at the end of the solution sampling, we collected some samples in smaller periods, so that it is possible to visualize with more accuracy the real moment plants reach  $C_{\min}$ .

### 3.4. Physiological parameters

'Paulsen 1103' rootstock presented higher intercellular  $\text{CO}_2$  concentration, compared to 'Magnolia' rootstock (Fig. 5c) resulting in no  $\text{CO}_2$  availability limitation, which optimizes the functioning of photosynthesized C assimilation pathways, resulting in a higher  $\text{CO}_2$  fixation in leaf tissues, which may be related to the higher dry matter yield of 'Paulsen 1103' rootstock leaves (Table 1) (Martim et al., 2009; Tcherkez et al., 2017). The higher  $\text{NO}_3^-$  and  $\text{NH}_4^+$  influxes contributed to a greater N accumulation in 'Paulsen 1103' rootstock leaves (Table 1). This may also have contributed to the increase in photosynthesized C

assimilation, corroborating with Greer (2018), which observed an increase in  $\text{CO}_2$  assimilation rate in apple trees with higher N contents in leaves. However, N percentage in leaves was the same for both rootstocks (Table 1). Thus, the higher leaf growth contributed to the fact that there was no difference in  $\text{CO}_2$  net assimilation rate between rootstocks (Nadal and Flexas, 2019). Besides, this higher intercellular  $\text{CO}_2$  concentration may be due to increased respiration (Tcherkez et al., 2017). This elucidates 'Paulsen 1103' rootstock greater efficiency in  $\text{NO}_3^-$  and  $\text{NH}_4^+$  uptake and, consequently, the greater N accumulation in leaves, which carry out the photosynthesis process, mainly for its contribution in important chloroplastidic proteins composition (Blank et al., 2018; Moriwaki et al., 2019).

'Magnolia' rootstock presented higher water use efficiency compared to 'Paulsen 1103' rootstock (Fig. 5e). Although there was no difference in transpiration rate between rootstocks (Fig. 5d), which is expressed in  $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$ , that is, for the same unit of leaf area, the rootstocks did not differ statistically. However, as 'Paulsen 1103' rootstock presented a higher shoot production, which can be understood as a larger surface area, it presents a greater transpiration per plant than 'Magnolia'. Thus, the greater the water loss due to transpiration, the lower the water use efficiency (Fig. 5e). This is an adaptive strategy of plants, since when exposed to low water and nutrients availability conditions, its cells perform this physiological process, which allows grapevines development, for example, in water deficit regimes regions (Martim et al., 2009; Flexas et al., 2010; Wu et al., 2018). On the other hand, it may result in lower N absorption in its forms and, consequently, lower plant growth, as verified by lower increase in height and stem diameter in 'Magnolia' rootstock (Table 1). In addition, water use efficiency and maximum and minimum fluorescence are important indicators of stress in plants and demonstrate a possible ability to grow in different habitats (Wu et al., 2018). Both grapevine rootstocks showed no differences for the net photosynthetic



**Fig. 4.** Concentration of  $\text{NH}_4^+$  in nutrient solution, cultivated with 'Paulsen 1103' (a) and 'Magnolia' (b) rootstocks grown in Hoagland nutrient solution after 20 d of reduced internal nutrient reserves. \* Time reaching lowest concentration  $p < 0.05$ .

rate, stomatal conductance of water vapors, transpiration rate, and instantaneous Rubisco carboxylation efficiency (Fig. 5a, b, d, f).

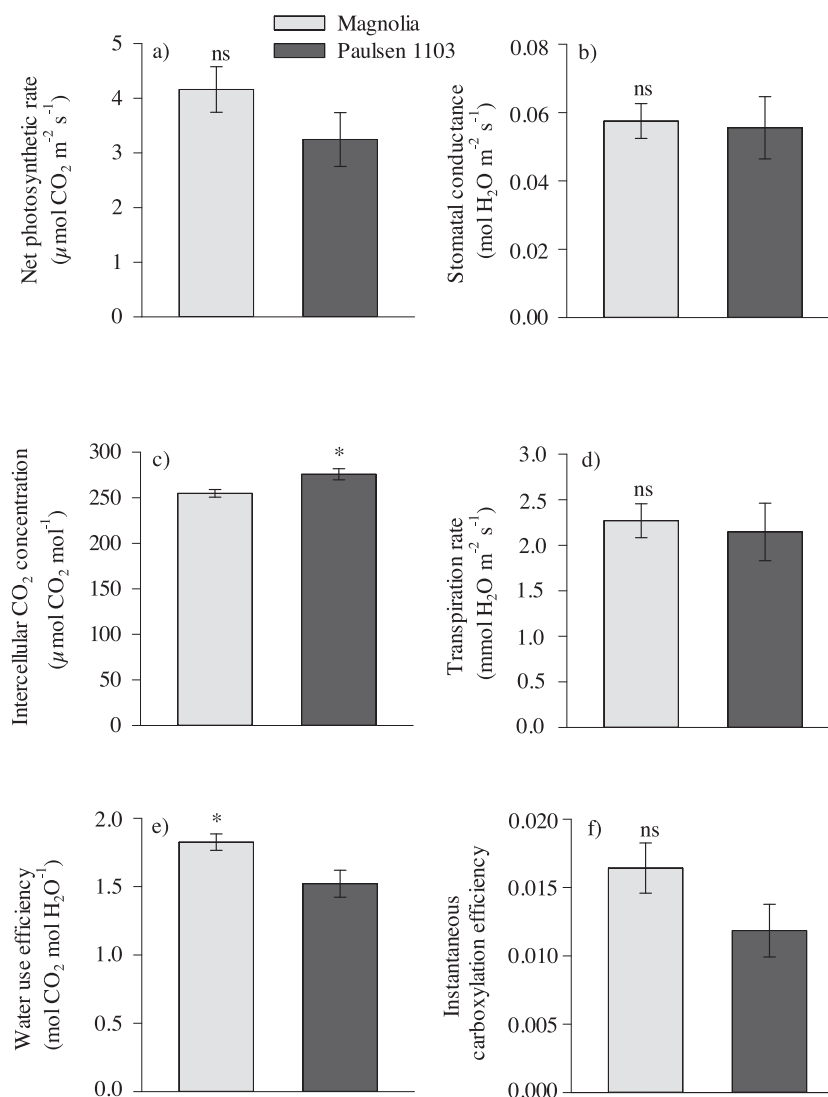
The light energy absorbed by chlorophyll molecules can result in different purposes, being used in photochemical processes in photosynthesis, dissipated as heat, or reissued as light, chlorophyll fluorescence (Maxwell and Johnson, 2000). 'Paulsen 1103' rootstock presented lower values of energy loss through minimum fluorescence ( $F_0$ ) and maximum fluorescence ( $F_m$ ) when compared to 'Magnolia' rootstock (Fig. 6). Thus, it is understood that a greater light energy proportion was destined for the photochemical reaction in this rootstock. This is evidenced by higher values of quantum yield of photosystem II ( $F_v/F_m$ ) and electron transport rate (ETR<sub>m</sub>) presented by 'Paulsen 1103' rootstock. The lower  $F_0$  and  $F_m$  values indicate a decrease in open reaction centers proportion (Maxwell and Johnson, 2000) and may be related to lower chlorophyll concentration values in leaves (Tiecher et al., 2016, 2017). The lower  $F_v/F_m$  and ETR<sub>m</sub> values for 'Magnolia' rootstock, suggest the stress occurrence on the photosynthesis process, mainly due to lower  $\text{NO}_3^-$  and  $\text{NH}_4^+$  uptake, represented by lower N forms influx by rootstock (Fig. 2). Also, the lower  $F_v/F_m$  and ETR<sub>m</sub> values show that a greater energy amount was dissipated via fluorescence by 'Magnolia' rootstock and, consequently, lower energy was used in photochemical processes of photosynthesis (Kalaji et al., 2016; Wu et al., 2018). On the other hand, 'Paulsen 1103' rootstock showed higher  $F_v/F_m$  and ETR<sub>m</sub> values associated to lower photochemical losses (Fig. 6), since the lower the  $F_0$  value, the lower the reduction-oxidation center PSII receptors and, the greater the excitation energy transfer from the light collecting system to the reaction center (Banks, 2017; Mascia et al., 2017). These results may be related to the higher N accumulation in leaves 'Paulsen 1103' rootstock, leading to a better N status in the plant, which allows greater chlorophylls synthesis, pigments responsible for light absorption and proteins important for the electron transfer chain in photosynthesis photochemical phase (Roca

et al., 2018).

### 3.5. Principal component analysis

Principal component analysis (PCA) was performed by extracting only the first two components, PC1 and PC2, as the sum of them explained 75.12 % of original data variability (Fig. 7a, b). Of these, 58.68 % were explained by principal component 1 and the remaining 16.44 % by principal component 2. Thus, PC1 separated 'Paulsen 1103' and 'Magnolia' plants in two groups with different behaviors. Variables with the greatest influence on the group formed by 'Paulsen 1103' rootstock replications were  $V_{\max}$  of  $\text{NH}_4^+$ , increase in height (Ih) and stem diameter (Isd); N accumulated in leaves (LNC), stem (CNS) and roots (RNC); leaf dry matter (LDM), stem (SDM), roots (RDM) and total (TDM); (rv), root length (rl), root volume (rv), quantum yield of photosystem II ( $F_v/F_m$ ), electron transport rate (ETR<sub>m</sub>) and intercellular  $\text{CO}_2$  concentration (Ci). On the other hand, 'Magnolia' plants had higher influences of the following variables:  $C_{\min}$  of  $\text{NO}_3^-$  and  $\text{NH}_4^+$ ,  $K_m$  of  $\text{NO}_3^-$  and  $\text{NH}_4^+$ , root/shoot ratio (R/S), minimum ( $F_0$ ) and maximum ( $F_m$ ) fluorescence, (W), liquid photosynthetic rate (E), stomatal conductance (Gs), transpiration rate (A), instantaneous carboxylation efficiency (A/Ci).

The PCA results show two data clusters, elucidating the differentiation between 'Paulsen 1103' and 'Magnolia' rootstocks (Fig. 7a). 'Paulsen 1103' rootstock clustering showed the best development and efficiency in N uptake, presenting an inverse relationship between  $C_{\min}$  and  $K_m$  of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  (Fig. 7b), that the lower its values, as the lower the minimum concentration at which roots can extract a nutrient from solution, greater the ion affinity to absorption sites of transporter and, therefore, more efficient in absorption will be the rootstock. From this, increases of N accumulated in leaves, stem and roots is observed, aiding in intercellular  $\text{CO}_2$  assimilation, provided by photosystem II



**Fig. 5.** Net photosynthetic rate (a), stomatal conductance (b), intercellular  $\text{CO}_2$  concentration (c), transpiration rate (d), water use efficiency (e) instantaneous carboxylation efficiency (f) of ‘Paulsen 1103’ and ‘Magnolia’ rootstocks grown in Hoagland nutrient solution after 20 d of reduced internal nutrient reserves. Vertical bars indicate mean and relevant asterisk at the top indicate statistical significant by Student's *t*-test. (\* = Significant by Student's *t*-test ( $p < 0.05$ ); \*\* = Significant by Student's *t*-test ( $p < 0.01$ ); ns = not significant).

best quantum yield and electron transport rate and, thus, greater root system development, such as length, surface area and root volume, contributing to increase in stem height and diameter and, consequently, higher dry matter yield of leaves, stem and roots, represented by the positive relationship of variables mentioned in the PCA. Another verified clustering shows the difference of ‘Magnolia’ rootstock (Fig. 7a), which had a positive relationship between  $C_{\min}$  of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  and,  $K_m$  of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  (Fig. 7b), showing the low affinity of roots absorption sites to ions in solution and consequent low ions absorption capacity in low concentrations in solution, possibly causing physiological stress to the rootstocks, observed by the positive relationship between minimum ( $F_o$ ) and maximum ( $F_m$ ) fluorescence, where a lower absorbed energy amount was observed and assimilated in photosynthetic processes.

#### 4. Conclusions

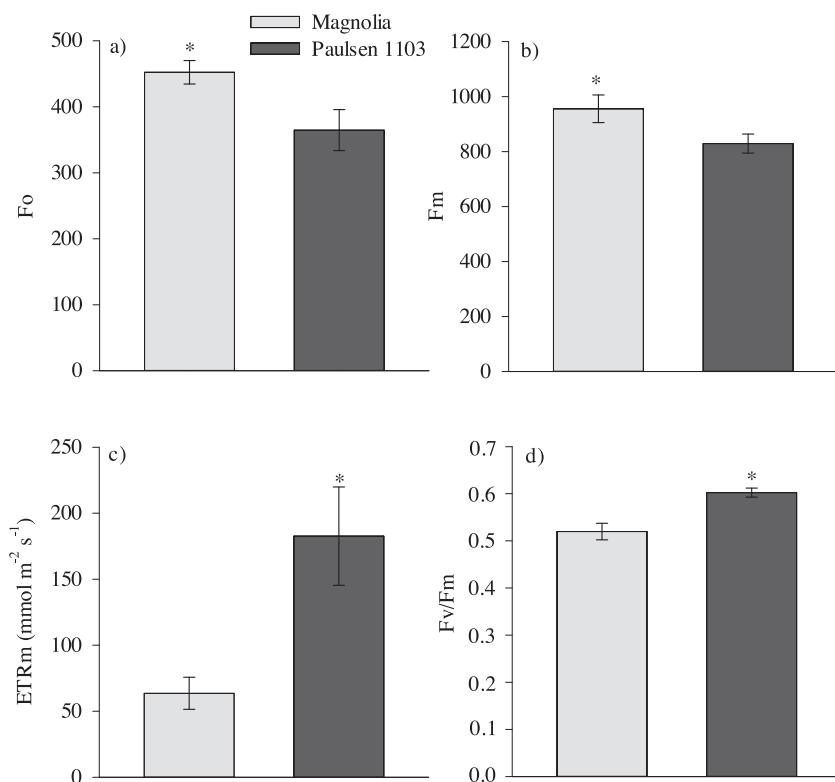
‘Paulsen 1103’ rootstock was the most efficient in  $\text{NO}_3^-$  and  $\text{NH}_4^+$  absorption, since it presented kinetic parameters such as lower  $C_{\min}$  and  $K_m$  values when compared to ‘Magnolia’ rootstock. Grapevine rootstocks for being selected in breeding programs should present lower

$C_{\min}$  and  $K_m$  values. Additionally, these kinetic parameters should correlate with root morphological parameters, such as root area, root volume and root length. However, kinetic gait studies of grapevine rootstocks should have a minimum evaluation period to reach  $\text{NO}_3^-$   $C_{\min}$  about 63 h and about 65 h for  $\text{NH}_4^+$ . Thus, kinetic parameters are important parameters for predicting N uptake efficiency in grapevine rootstocks and they can be an important tool for grapevine cultivars genetic improvement.

#### Author contributions section

Matheus Severo de Souza Kulmann led the study, performed the greenhouse work and laboratory analysis and wrote the paper; Paula Beatriz Sete, Wagner Squizani Arruda, Gabriel Alberto Sans, Carolina Flogiarini Parciannello and Betania Vahl de Paula participated on plant growing and  $\text{NO}_3^-$  and  $\text{NH}_4^+$  determination in solution and N in plants and also contributed to the data discussion; Lincon Oliveira Stefanello and Rai Augusto Schwalbert contributed to the data analysis and writing of the paper; Raissa Schwalbert contributed to the photosynthetic analysis and data discussion; Fernando Teixeira Nicoloso, George Wellington Bastos de Melo and Henrique Pessoa dos Santos





**Fig. 6.** Initial fluorescence (Fo) (a), maximum fluorescence (Fm) (b), electron transport rate (ETRm) (c) and maximum quantum yield of PSII (Fv/Fm) (d) in leaves of 'Paulsen 1103' and 'Magnolia' rootstocks grown in Hoagland nutrient solution after 20 d of reduced internal nutrient reserves. Vertical bars indicate mean and relevant asterisk at the top indicate statistical significant by Student's *t*-test. (\* = Significant by Student's *t*-test ( $p < 0.05$ ); \*\* = Significant by Student's *t*-test ( $p < 0.01$ ); ns = not significant).

participated on plant growing and data discussion; and Gustavo Brunetto led the study and contributed to the data discussion and writing of the paper.

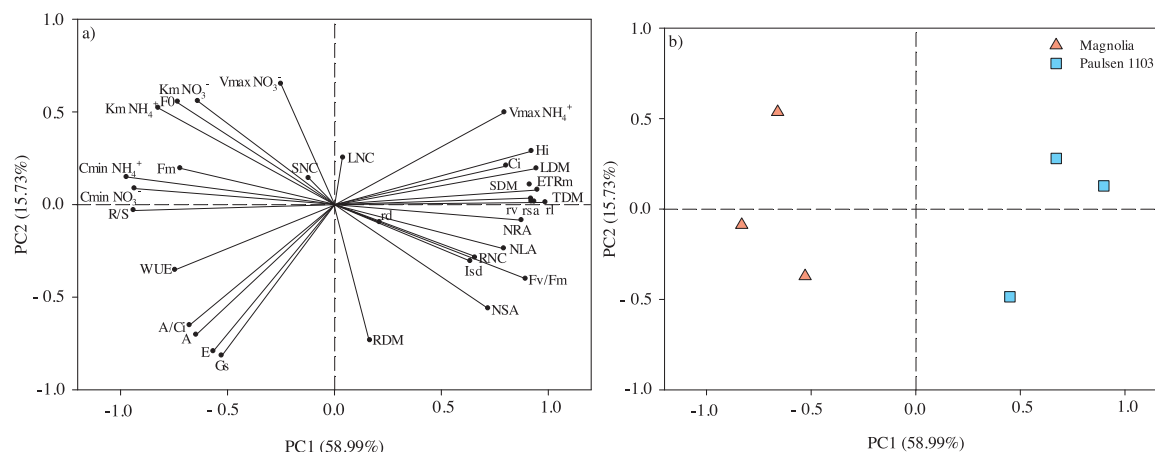
#### Declaration of Competing Interest

The authors declare no financial or other competing conflicts of interest.

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**Fig. 7.** Relationship between principal component 1 (PC1) and principal component 2 (PC2) for variable groups of (a) kinetic parameters of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  ( $V_{\max}$ ,  $K_m$ ,  $C_{\min}$ ), morphological (increase in height (Hi), increase in stem diameter (Isd), leaf dry matter (LDM), stem dry matter (SDM), root dry matter (RDM), total dry matter (TDM), root/shoot ratio (R/S), total N in leaves (LNC), total N in stems (SNC), total N in roots (RNC), N accumulated in leaves (NLA), N accumulated in stem (NSA), N accumulated in roots (NRA)), root morphological parameters (root surface area (rsa), root volume (rv), root diameter (rd), root length (rl)) and physiological parameters (initial fluorescence (Fo), maximum fluorescence (Fm), electron transport rate (ETRm), maximum quantum yield of PSII (Fv/Fm), net photosynthetic rate (E), stomatal conductance (Gs), intercellular  $\text{CO}_2$  concentration (Ci), transpiration rate (A), water use efficiency (WUE) instantaneous carboxylation efficiency (A/Ci)) of (b) 'Paulsen 1103' and 'Magnolia' rootstocks grown in Hoagland nutrient solution after 20 d of reduced internal nutrient reserves.

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.scienta.2020.109174>.

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